Reaction of N-*p*-Tolyldiphenylacetimide (III, R = $(C_6H_5)_2CH$) **with *p*-Toluidine.**—A solution of 0.4 g. (0.8 millimoles) of the imide III (R = $(C_6H_5)_2CH$) and 0.8 millimole of *p*-toluidine in 15 ml. of toluene was refluxed for 80 hours and then evaporated to dryness *in vacuo*. The resulting white solid, m.p. 169–172°, was recrystallized from 95% ethanol to yield 0.3 g. (61%) of N-(*p*-tolyl)-diphenylacetamide (VII), m.p. 179–180°.

Reaction of Adduct XII with *p*-Toluidine.—A solution of 3.6 g. (20 millimoles) of ethyl-*n*-butylketene *n*-butylimine[7] and 8.5 g. (40 millimoles) of diphenylacetic acid in 60 ml. of dioxane was refluxed for 12 hours and then evaporated to dryness *in vacuo*. The residue was dissolved in 100 ml. of ethyl acetate and washed three times with a saturated sodium bicarbonate solution, two times with water and finally two times with saturated sodium chloride solution. The ethyl acetate solution was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness *in vacuo* to yield 6.57 g. (84%) of the oily diphenylacetic acid adduct of the ketenimine XII.

The adduct was dissolved in 50 ml. of dioxane and refluxed for 50 hours with 10.0 g. (0.1 mole) of *p*-toluidine. The clear solution was evaporated to dryness *in vacuo* to yield an oily residue which was dissolved in 150 ml. of ethyl acetate and washed three times with 3 *N* hydrochloric acid, two times with water and finally two times with a saturated solution of sodium chloride. The ethyl acetate solution was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness *in vacuo*. Petroleum ether (30–60°) was added to the oily residue and the contents were cooled overnight at 4°. The resulting crystalline solid was collected by filtration and washed with cool petroleum ether to yield 1.35 g. (27% based on the adduct) of N-(*p*-tolyl)-diphenylacetamide, m.p. 167–169°. Recrystallization from hexane–acetone raised the melting point to 172–173° and a mixture melting point with authentic amide was not depressed.

Acknowledgment.—The authors wish to thank Dr. J. M. Vandenbelt, R. B. Scott and their associates at Parke, Davis and Co. for the infrared specral determinations. They are particularly indebted to Professor Ch. Prévost, in whose laboratories at the Sorbonne much of this work was done, for his kindness and hospitality. The aid of the John Simon Guggenheim Memorial Foundation also is gratefully acknowledged.

DETROIT 2, MICHIGAN

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF WAYNE STATE UNIVERSITY]

Nitrogen Analogs of Ketenes. V.[1] Formation of the Peptide Bond

BY CALVIN L. STEVENS AND MORTON E. MUNK[2,3]

RECEIVED MARCH 7, 1958

Nitrogen analogs of ketenes have been used as reagents for the formation of the peptide bond. The N-protected amino acids glycine, alanine and methionine were converted in high yield to stable, crystalline adducts with the ketenimine I. The adducts were formed in non-polar, aqueous or alcoholic solutions. Reaction of an amino acid ester with an adduct afforded the peptides; alternately, the three starting materials could be placed in the reaction mixture at the same time. *p*-Aminobenzoic acid ethyl ester was acylated in good yield. Dipeptide derivatives of L-cysteinyl-L-tyrosine and L-asparaginyl-L-cysteine were prepared in about 30 and 45% yield, respectively, indicating that the phenol of tyrosine need not be protected and that dipeptides of good optical purity could be obtained by this method.

In the previous paper[1] in this series, the nitrogen analogs of ketenes were found to react with carboxylic acids with the formation of imides and these imides were found to be active acylating agents. In view of the striking success of carbodiimines[4] as reagents for the formation of peptides, the purpose of this investigation was to determine the use of the ketenimines for the same purpose.

The reaction of phthaloylglycine (II) with the ketenimine I gave the adduct III in 92% yield. The adduct was purified by recrystallization and was stable. Similar adducts from N-carbobenzoxyglycine, phthaloyl-β-alanine, and phthaloyl-DL-methionine were prepared in 89, 76 and 60% yields, respectively. An inert solvent was satisfactory for the adduct formation, although ethanol–water and dioxane–water reaction mixtures could be used.

Acylation of glycine ethyl ester with the adduct II produced the dipeptide derivative, phthaloylglycylglycine ethyl ester, in 70% yield. In the presence of an appropriate base, amino acid or peptide ester hydrochlorides could be used as the starting materials. The tripeptide derivative, phthaloylglycylglycylglycine ethyl ester, was prepared in 50% yield from the adduct III and glycylglycine ethyl ester hydrochloride in the presence of triethylamine.

Ethyl *p*-aminobenzoate was used to test the ability of the adduct III to acylate an aromatic amine. After reaction in benzene solution the product, ethyl phthaloylglycyl-*p*-aminobenzoate, was isolated in 73% yield. A similar reaction was performed in methylene chloride in which the three starting materials ketenimine I, phthaloylglycine and ethyl *p*-aminobenzoate were placed in the reaction mixture at the same time. The yield of product was 77%. In view of the mechanism of the reaction of ketenimines with carboxylic acids as described in the previous paper,[1] the acylation of the amino acid ester in this latter reaction probably proceeded in part *via* the isoimide adduct.

In each of the reactions described so far, the by-product amide V was separated easily from the peptide derivative by taking advantage of solubility differences in various solvents. In other examples the separation problem was not as easily solved. In these examples the peptide ester was selectively saponified and removed as a carboxylic acid. In the preparation of phthaloylglycyl-L-leucine, the reaction mixture was subjected to acid

(1) Part IV describes the reaction of ketenimines with carboxylic acids and is in THIS JOURNAL, **80**, 4065 (1958).

(2) Supported by a Parke-Davis Research Fellowship.

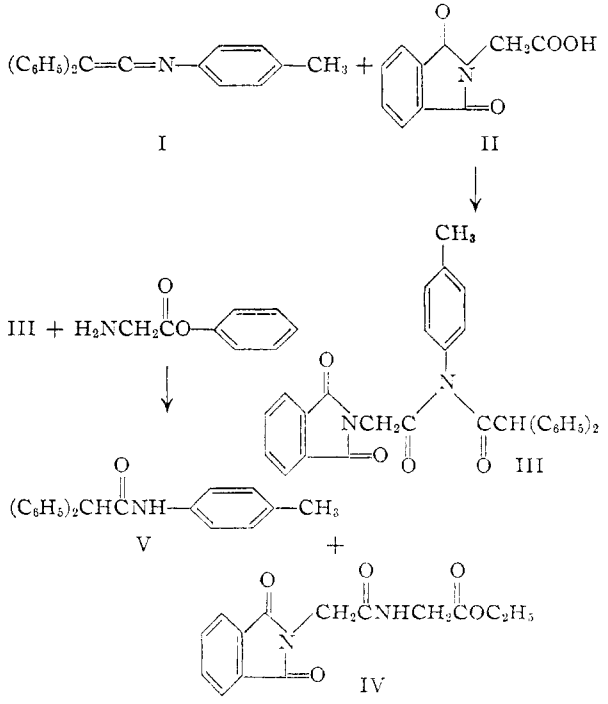
(3) Abstracted from the dissertation submitted by M. E. Munk in partial fulfillment of the requirement for the degree of Doctor of Philosophy, Wayne State University, 1957.

(4) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955), and later papers; L. Velluz, G. Amiard, J. Bartos, B. Goffinet and R. Heymes, *Bull. soc. chim. France*, 1404 (1956).

hydrolysis from which the acid was isolated in 30% yield.

In the synthesis of N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine, the intermediate adduct from the reaction of the ketenimine I and N-carbobenzoxy-S-benzyl-L-cysteine was not isolated but further treated *in situ* with L-tyrosine methyl ester hydrochloride in the presence of triethylamine. The resulting mixture of dipeptide methyl ester and the amide V was treated with a dilute sodium hydroxide solution at room temperature. Removal of the unreacted amide VII followed by acidification of the basic solution resulted in a 30% yield (based on the ketenimine I) of the dipeptide. The specific rotation was slightly higher than that recorded in the literature,[5] but in our opinion the best criterion for the optical purity of this product was the sharp melting point after repeated recrystallizations. The formation of the peptide also demonstrates that a phenolic group need not be protected in this method.

N - Carbobenzoxy - L - asparaginyl - S - benzyl-L-cysteine methyl ester also was synthesized without isolation of the intermediate adduct. The dipeptide methyl ester easily was separated from the amide by fractional crystallization. The specific rotation of the product was essentially the same as that previously reported[5] by two other methods and the yield was 45%.



This method of peptide synthesis is similar to the various mixed anhydride methods[6] as well as the carbodiimide method. The potential advantage of the present mixed imide method is that active acylating agents for each amino acid can be isolated, purified and stored. The present method suffers the same disadvantage as do other mixed anhydride methods since the two carbonyl groups of the mixed imide adduct are chemically very similar and the possibility exists of a significant amount of reaction at the wrong carbonyl. However, the previous investigation[1] has shown that the direction of attack upon the imides is subject to electronic as well as steric control. Further, in a reaction mixture of acid, amine and ketenimine I any amount of acylation which proceeds *via* the isoimide intermediate will almost certainly give only peptide and amide V.

(5) R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud and J.-P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

(6) R. A. Boissonas, *ibid.*, **34**, 874 (1951); J. R. Vaughan, THIS JOURNAL, **73**, 3547 (1951); Th. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951). G. W. Kenner has recently reviewed the use of mixed anhydrides (*Chem. Soc. Special Publ. No.* **2**, 103 (1955) and M. Bodanszky recently has discussed the general implications of activated ester syntheses in the *Record Chem. Progr.*, **18**, 187 (1957).

Experimental

Reaction of Diphenylketene-*p*-tolylimine (I) with Phthaloylglycine. Procedure A.—A solution of 2.0 g. (7.1 millimoles) of the ketenimine I and 1.5 g. (7.1 millimoles) of phthaloylglycine in 35 ml. of benzene was refluxed until the yellow ketenimine color was discharged completely and then evaporated to dryness *in vacuo*. The solid residue, m.p. 168–170°, was recrystallized from hexane–acetone to yield 3.2 g. (92%) of the N-phthaloylglycyldiphenylacetic acid *p*-toluide (III), m.p. 179.5–180.5°. Further recrystallization from hexane–acetone did not raise the melting point.

Procedure B.—A solution of 0.44 g. (2.12 millimoles) of phthaloylglycine in 5 ml. of 95% ethanol was added to a solution of 0.6 g. (2.12 millimoles) of the ketenimine I in 10 ml. of 95% ethanol. Gentle heating was necessary to dissolve the ketenimine in 95% ethanol. After the solutions were mixed together, 3 ml. of water was added dropwise with swirling. Although a fine precipitate formed after a short period, the contents were allowed to remain at room temperature for 48 hours. At the end of this time the yellow ketenimine color was discharged and the white solid was filtered to yield 0.45 g. (44%) of the adduct III, m.p. 177–179°.

Procedure C.—To the clear yellow solution of 0.6 g. (2.12 millimoles) of the ketenimine I and 0.45 g. (2.12 millimoles) of phthaloylglycine in 11 ml. of dioxane was added 1 ml. of water. After 66 hours at room temperature the pale yellow solution was poured onto ice. The resulting solid was filtered, washed with cold water, with a saturated sodium bicarbonate solution, again with cold water, and then dried to yield 0.92 g. (92%) of impure phthaloylglycine adduct, m.p. 165–170°. Recrystallization from hexane–acetone produced 0.68 g. (66%) of the adduct III, m.p. 179.5–180.5°.

Anal. Calcd. for $C_{31}H_{24}O_4N_2$: C, 76.20; H, 4.95. Found: C, 76.00; H, 5.14.

Preparation of Other Adducts.—Following procedure A, the N-carbobenzyloxyglycine adduct could be prepared in 89% yield. From 8.0 g. (28 millimoles) of ketenimine I and 5.9 g. (28 millimoles) of N-carbobenzyloxyglycine was obtained 12.35 g. of the adduct, m.p. 166–167°, after recrystallization from benzene–petroleum ether.

Anal. Calcd. for $C_{31}H_{28}O_4N_2$: C, 75.58; H, 5.73. Found: C, 75.57; H, 5.77.

The phthaloyl-β-alanine adduct could be prepared in 76% yield, m.p. 121.5–122.5°, after recrystallization from ethyl acetate–petroleum ether.

Anal. Calcd. for $C_{32}H_{26}O_4N_2$: C, 76.47; H, 5.22. Found: C, 76.51; H, 5.47.

The phthaloyl-DL-methionine adduct could be prepared in 60% yield using tetrahydrofuran as a solvent, m.p. 135–136°, after recrystallization from methanol.

Anal. Calcd. for $C_{34}H_{30}N_2O_4S$: C, 72.58; H, 5.38. Found: C, 72.59; H, 5.56.

Phthaloylglycylglycine Ethyl Ester (IV).—A solution of 1.0 g. (2.1 millimoles) of the phthaloylglycine adduct III and 0.2 g. (2.1 millimoles) of ethyl glycinate, b.p. 66–67° (40 mm.), $n^{20}D$ 1.4210, in 20 ml. of benzene was refluxed for 3 hours. The resulting white crystalline solid was filtered from the hot solution and washed with benzene to give 0.18 g. of phthaloylglycylglycine ethyl ester,[7] m.p. 188–189°.

(7) S. Goldschmidt and H. Lautenschlager, *Ann.*, **580**, 68 (1953), report m.p. 190°.

The combined filtrate and washings were evaporated to dryness *in vacuo* and the resulting solid residue was extracted several times with boiling water. Cooling the water solution produced an additional 0.17 g. of the dipeptide to raise the yield to 59%. The residue remaining after the water extraction was recrystallized from ethanol–water to yield 0.42 g. (68%) of N-(*p*-tolyl)-diphenylacetamide (IV), m.p. 167–169°. A mixture melting point with an authentic sample was not depressed, m.p. 168–170°.

Ethyl Phthaloylglycyl-*p*-aminobenzoate. Method A.—A solution of 1.0 g. (2.1 millimoles) of the phthaloylglycine adduct III and 0.34 g. (2.1 millimoles) of ethyl *p*-aminobenzoate in 20 ml. of benzene was refluxed for 44 hours and then slowly cooled to room temperature. The resulting white crystalline solid was filtered, washed with cold benzene, and dried to give the dipeptide, m.p. 190–200°. Two recrystallizations from benzene produced 0.25 g. of the dipeptide, m.p. 207–208°. Slight concentration and cooling of the combined filtrate and washings produced an additional 0.27 g. of the dipeptide, m.p. 206–207°, to raise the yield to 73%.

Method B.—To 15 ml. of methylene chloride was added 1.0 g. (3.5 millimoles) of diphenylketene-*p*-tolylimine (I), 0.8 g. (3.9 millimoles) of phthaloylglycine and 0.6 g. (3.6 millimoles) of ethyl *p*-aminobenzoate. The contents were refluxed for 1 hour and 40 minutes, although the yellow ketenimine color was completely discharged after 15 minutes. The solution was evaporated to dryness *in vacuo* and the solid residue was dissolved in hot benzene, from which crystallized 0.95 g. (77%) of the dipeptide, m.p. 204.0–205.5°.

Anal. Calcd. for $C_{19}H_{16}N_2O_5$: C, 64.76; H, 4.58. Found: C, 64.48; H, 4.63.

Phthaloylglycylglycylglycine Ethyl Ester.—To a solution of 1.0 g. (2.1 millimoles) of the phthaloylglycine adduct III in 10 ml. of methylene chloride was added, with stirring, 0.4 g. (2.1 millimoles) of glycylglycine ethyl ester hydrochloride, m.p. 171–173°, and 0.5 ml. (3.3 millimoles) of triethylamine. The contents were heated for 9 hours with continuous stirring. During this period the amount of suspended solid gradually increased. The white solid was then filtered, washed with cool water, and dried to give 0.35 g. (49%) of the tripeptide, m.p. 221–222°. Recrystallization from water raised the melting point to 225–226°. Hofmann reported a melting point of 228–230° for the tripeptide.[8]

Phthaloylglycyl-L-leucine.—A solution of 1.0 g. (2.1 millimoles) of the phthaloylglycine adduct III and 0.34 g. (2.1 millimoles) of ethyl L-leucinate, b.p. 70° (5.0 mm.), $n^{25}D$ 1.4268, in 15 ml. of benzene was refluxed for 2 hours and then evaporated to dryness *in vacuo*. To the solution of the resulting solid in 20 ml. of acetone was added 6.0 ml. of water and 2.5 ml. of concentrated hydrochloric acid. The contents were refluxed for 2 hours and then evaporated to dryness *in vacuo*. The residue was triturated with 20 ml. of 10% potassium bicarbonate solution. After filtration the basic solution was carefully acidified with concentrated hydrochloric acid to the congo red end-point. The resulting solid was filtered and recrystallized from ethanol–water to yield 0.18 g. (28%) of the dipeptide, m.p. 104–106°. The reported[9] melting point was 105–106°.

(8) K. Hofmann, A. Lindenmann, M. F. Magee and N. H. Khan, THIS JOURNAL, **74**, 470 (1952).

(9) K. Yamashita, *J. Agr. Chem. Soc. Japan*, **28**, 672 (1954).

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine.—A solution of 0.6 g. (2.1 millimoles) of diphenylketene-*p*-tolylimine (I) and 0.75 g. (2.2 millimoles) of N-carbobenzoxy-S-benzyl-L-cysteine in 15 ml. of methylene chloride was refluxed until the yellow ketenimine color was discharged (1 hour). In the cooled solution was suspended 0.51 g. (2.2 millimoles) of tyrosine methyl ester hydrochloride. After 0.3 ml. (2.3 millimoles) of triethylamine was added, the amino acid ester hydrochloride slowly dissolved. A turbid solution remained, which was refluxed for 3 hours, filtered, and then evaporated to dryness *in vacuo*. The viscous oil that remained was dissolved in 13 ml. of methanol and to this solution 1.7 ml. of 4 *N* sodium hydroxide was added dropwise. A copious precipitate formed and the contents were shaken for 75 minutes. After the addition of 2 ml. of water, the solid was filtered and washed with a few ml. of water. Recrystallization of a small portion of the solid yielded pure N-(*p*-tolyl)-diphenylacetamide (V), m.p. 171–172°.

Acidification of the cooled filtrate with 7 ml. of 1 *N* hydrochloric acid produced an oil. Addition of 30 ml. of water resulted in further oil. The supernatant liquid was decanted carefully and the oily residue was washed with a small amount of cold water. The oil was crystallized from ethanol–water to yield a solid, m.p. 184–187°. Recrystallization from ethyl acetate–petroleum ether produced 0.33 g. (32%) of the dipeptide, m.p. 196.0–196.5°, $[\alpha]^{23}D$ +6.68° (*c* 2, 1 *N* NaOH). The value $[\alpha]^{21.5}D$ +5.45° (*c* 2, 1 *N* NaOH) and m.p. 198–200° have been reported.[5]

N-Carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteine Methyl Ester.—A solution of 0.5 g. (1.77 millimoles) of diphenylketene-*p*-tolylimine (I) and 0.5 g. (1.87 millimoles) of N-carbobenzoxy-L-asparagine in 28 ml. of tetrahydrofuran was refluxed for 23 hours. The slight amount of residue present in the pale yellow solution was filtered and the solution was concentrated to a volume of 20 ml. With stirring, 0.47 g. (1.80 millimoles) of S-benzyl-L-cysteine methyl ester hydrochloride and 0.28 ml. (2.0 millimoles) of triethylamine were added to this solution. After a few minutes only a finely divided solid remained. The reaction was stirred at room temperature for 22 hours and then refluxed for an additional 2 hours. The amount of suspended solid present gradually increased. The solid was filtered, washed with tetrahydrofuran, then water, and finally recrystallized from pyridine–water to yield 0.33 g. (41%) of the dipeptide, m.p. 192–193°, $[\alpha]^{23}D$ −31.2° (*c* 2, pyridine). The reported[5] constants are m.p. 189 and 196°, $[\alpha]^{18}D$ −31.5° (*c* 2.3, pyridine) and $[\alpha]^{19}D$ −31.9° (*c* 2.4, pyridine). Recrystallization from pyridine–water raised the melting point to 195.0–195.5°. From the mother liquors an additional 0.04 g. of the dipeptide was recovered raising the yield to 45%.

Acknowledgment.—The authors wish to thank Mr. C. C. Childs and associates at Parke, Davis and Co. for the analytical determinations. They are particularly indebted to Professor Ch. Prévost, in whose laboratory at the Sorbonne much of this work was done, for his kindness and hospitality. The aid of the John Simon Guggenheim Memorial Foundation also is gratefully acknowledged.

DETROIT 2, MICHIGAN